Keystone Journal of Undergraduate Research 2(1): 15-21. 2014

Automation of Discrimination Training for Cuttlefish (Mollusca: Cephalopoda)

Alexander Ryan Hough

Faculty Mentor:
[1]Dr. Jean Geary Boal
Department of Biology
Millersville University

ABSTRACT

Cephalopods are common subjects of learning experiments, yet discrimination stimuli are commonly presented by hand, which is both laborious and rife with opportunity for cuing. The following experiment tested the possibility that cuttlefish training could be automated using stimuli presented via computer monitor and food rewards presented in a food hopper. A single adult female common cuttlefish (*Sepia officinalis*) was trained first to attack a black rectangle (S+) for a live crab prey item and ignore a white right-angle (S-). Stimuli were then presented behind a clear Plexiglas partition and the cuttlefish was rewarded for attacking the Plexiglas in front of the S+. A food hopper was introduced to improve the delivery of the food reward. Finally, stimuli were presented on a computer monitor (CRT) located outside the tank and the cuttlefish was rewarded for attacking the Plexiglas in front of the S+ image. The cuttlefish was successful in learning the discrimination and in transferring learning from the physical objects to the computer images. Results indicate that automation of training using computer presentations of stimuli and automated food rewards is possible for cuttlefish.

Keywords: behavior; comparative cognition; flicker fusion; vision

The Coleoid cephalopods (octopuses, cuttlefishes, and squids) possess the most advanced nervous system within the phylum Mollusca and the largest brain of any invertebrate (Hanlon and Messenger 1996; Hickman et al. 2006). They are capable of complex behavior analogous to that of vertebrates (Hochner et al. 2003) and have been the subjects of a significant body of learning research (Hanlon and Messenger 1996).

Vertebrates are typically trained with automated presentations of images and then are rewarded using automated reward systems. Nearly all discrimination learning experiments with cephalopods have involved hand presentations of stimuli (reviewed in Boal 1996; e.g. Darmaillacq et al. 2008), necessitating considerable time and effort on the part of investigators as well as providing multiple opportunities for inadvertent cuing (Bitterman 1975; Papini and Bitterman 1991). Partly because of these problems, most recent research addressing cephalopod learning has focused on spatial learning using mazes (e.g. Cartron et al. 2012). The objective of this study was to assess the feasibility of using computer-run stimulus presentation and automated rewards to study discrimination learning in cephalopods.

The vision of Coleoid cephalopods is remarkably vertebrate-like (Hanlon & Messenger 1996), with acuity similar to that of fishes (Muntz and Gwyther 1988). Most cephalopod eyes possess only one type of visual pigment (Tansley 1965); as a result, they cannot see color (Roffe 1975; Flores et al. 1978; Flores 1983; Marshall and Messenger 1996; Mäthger et al. 2006), but they can discriminate between objects based on size, orientation, brightness, form, and plane of polarization (Shashar and Cronin 1996; Shashar et al. 2002; Horváth and Varjú 2004). A few cephalopods, including cuttlefish, are capable of depth perception due to converging eye movements (Messenger 1977). Most cephalopod eyes do not have a fovea, but cuttlefish and some bottom dwelling

[1]Corresponding author email: Jean.Boal@millersville.edu

cephalopods do, which improves their ability to focus (Young 1963).

Images that are presented on either a CRT computer monitor or LCD monitor typically display images with a refresh rate of 60 Hz (Menozzi et al. 2001). If an organism's flicker fusion threshold is greater than the refresh rate in frames per second, then they will perceive the images to flicker on and off, and cannot be expected to respond to the images in the same way as they would to the actual objects. Among cephalopods, the flicker fusion threshold is known only for *Octopus*, whose threshold is less than 30 Hz (Bullock and Budelmann 1991), considerably lower than has been documented in vertebrates (humans: 60 Hz; Gleitman 1992; chickens: 87 Hz; Lisney et al. 2011). Based on the evidence provided, it is reasonable to suppose, then, that octopuses and cuttlefishes could perceive images on a computer monitor without perceiving a flicker.

In the following experiment, I investigated if *Sepia officinalis* is capable of learning to detect and discriminate between computer images by using a protocol analogous to a touch screen, with the addition of a food hopper to reduce cuttlefish-experimenter interactions.

METHODS

Subject

Juvenile cuttlefish (*Sepia officinalis*; approximately 2 months post-hatching) were obtained from the National Resource Center for Cephalopods (NRCC) in Galveston, TX, shipped to Millersville University's Cephalopod Research Laboratory and reared to adulthood. At the time of this experiment, all but one cuttlefish had died of senescence. No further cuttlefish could be obtained because the NRCC, the sole source for all research cuttlefish in North America, closed after incurring heavy damage from Hurricane Rita. This last cuttlefish (a female, approximately 12 months post-hatching, 360 g, and 13 cm mantle length) served as the single experimental subject. She was fed live fiddler crabs as food rewards during trials and a 2 cm length section of thawed frozen shrimp each evening, after training.

Apparatus

The cuttlefish was housed and trained in a clear Plexiglas tank (40 cm x 30 cm x 38 cm deep). The front side of the tank had an extended overflow area of 6 cm, which was separated from the main living space by a Plexiglas divider. Water could enter the overflow area through a row of small holes positioned 3 cm from the top of the divider (Figure 1). The tank was furnished with a substrate of crushed oyster shells with several artificial plants and the seawater was part of a recirculating 5500-L system. Seawater was mixed from Instant Ocean brand sea salt and reverse-osmosis filtered water. All water passed through mechanical, chemical, and biological filters, a UV sterilizer, and a water chiller. Water was maintained at 18-20°C with a salinity level of approximately 35 ppt.

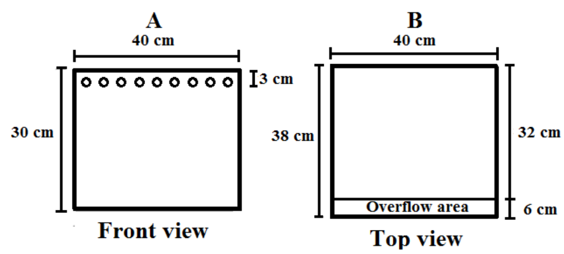

Figure 1. Schematic of the testing aquarium. A. The tank as viewed from the front, with height and width specifications as shown. B. The tank as viewed from above, with depth, width and size of the overflow area as shown.

The food hopper consisted of a piece of clear PVC pipe (3 cm internal diameter x 75 cm long), positioned directly above a round glass feeding dish (11 cm diameter x 5 cm deep) (Figure 2). To deliver a food reward, a live adult fiddler crab (*Uca* spp., carapace approximately 3 cm in diameter) was placed into the top of the PVC pipe and flushed down into the feeding dish with about a liter of water.

Two discrimination stimuli were used: a black Plexiglas rectangle (S+; 9 ½ cm high x 6 cm wide x 1 cm deep) and a white PVC right-angle (S-; 6 ½ cm high x 6 cm long x 2 ½ cm diameter) (Figure 3). Each stimulus was attached to the end of a clear acrylic rod (45 cm in length x ¾ cm diameter). In later trials, a computer monitor (Dell 48 cm CRT) was placed directly against the front of the tank

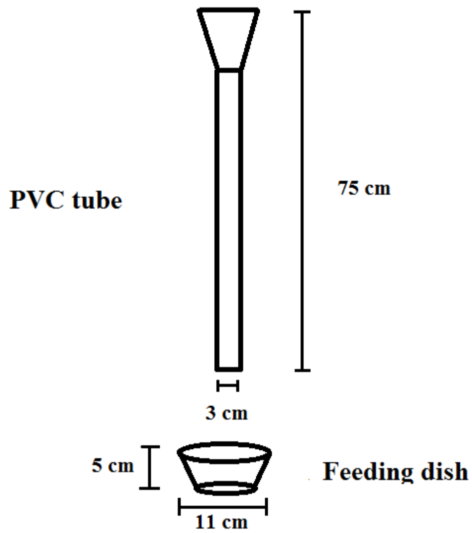

Figure 2. Schematic of the food hopper. Dimensions of the PVC tube and feeing dish are displayed (Not drawn to scale).

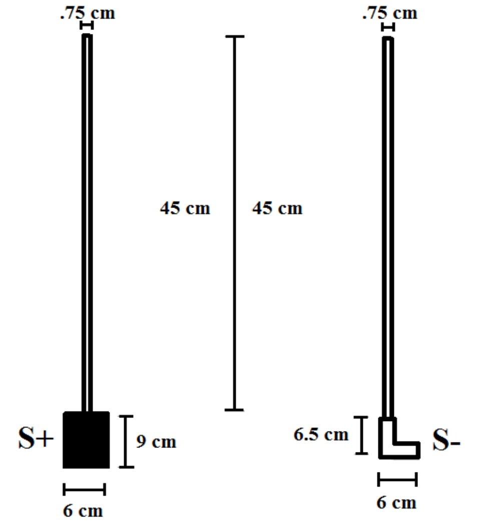

Figure 3. Discrimination stimuli. Both S+ (left) and S- (right) are shown with their corresponding dimensions.

and images of the stimuli were presented the same way they would be perceived when using hand presentations (to scale and including 9 cm of the stick), using Microsoft PowerPoint software. Images consisted of (i) photographs of the S+ and S-, placed on a solid, neutral gray background (Figure 4), (ii) the same neutral background with no stimuli, and (iii) an image of *Fucus* seaweed. A digital video camera (Canon 2R70MC) was used to record trials.

Procedure

At the start of each session, towels were draped over the sides of the tank to reduce visual distractions, the computer monitor was set up (later trials, only) and the cuttlefish was given ten minutes to acclimate. In all trials, the cuttlefish was only rewarded for striking or pressing against the Plexiglas in front of the S+. If the cuttlefish responded correctly to the S+, a live crab (*Uca* spp.) was immediately provided (directly into the home tank in early trials and into the food hopper in later trials), the stimuli were removed, and the response time was recorded. If the cuttlefish did not respond within 60 s, a "no response" was recorded and the trial ended. If the cuttlefish struck or pressed against the Plexiglas in front of the S-, the trial was ended, marked as unsuccessful, and the response time was recorded. There was at least a 30 minute interval between trials to allow for the complete consumption of the crab. Three trials were conducted per day in all conditions.

When images of stimuli were used in the place of the physical stimuli (later trials), training sessions began by turning on the computer monitor, which displayed an image of *Fucus* spp. seaweed. The seaweed image was chosen to indicate the beginning of trials because it was a complex, non-threatening natural image, distinct from the experimental stimuli. At the initiation of each trial, the screen was changed to a blank screen (neutral gray; to direct attention) for five seconds before displaying the stimulus slides (S+ or S-; S+ and S-). After a successful trial, or 60 seconds (whichever was shorter), the image of seaweed was again displayed on the screen.

Shaping and training were conducted in ten steps:

1. ***Shaping S+ with crab.*** In the first 10 trials, the S+ was presented alone, inside the housing tank, with a crab suspended on clear monofilament fishing line (5.5 kg test) directly in front of the S+. When the cuttlefish attacked the crab, the crab was released to the cuttlefish and the S+ was removed.

2. ***S+ with crab.*** In the next 45 trials, the S+ was presented with a crab, as above, but the stimuli were presented in the overflow area of the tank. If the cuttlefish attacked the Plexiglas in front of the S+, a crab was dropped into the cuttlefish tank, in clear view of the cuttlefish.

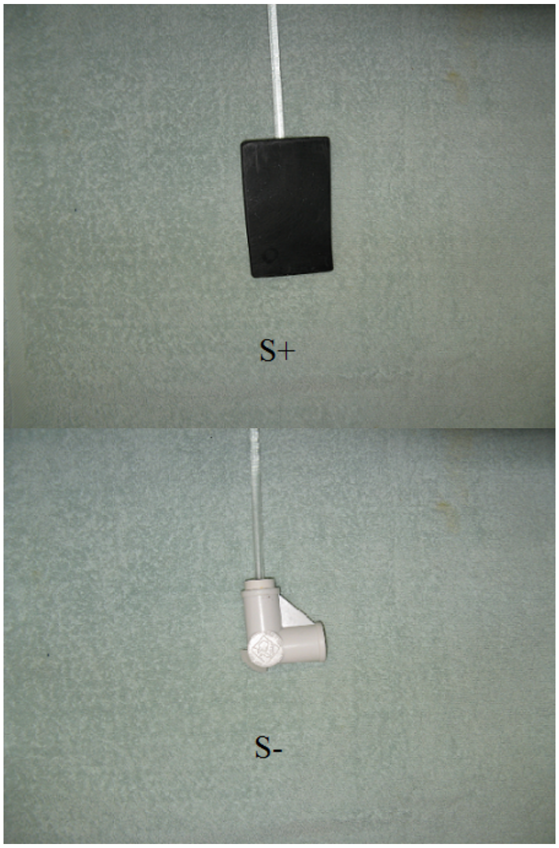

Figure 4. Discrimination stimuli. These photographs of both S+ and S- were incorporated into PowerPoint slides and were presented to the cuttlefish on a computer screen.

3. ***S+ alone.*** 45 further trials were conducted, as in condition A, except that the S+ was presented alone, without a crab. The cuttlefish was rewarded in the same way as in Condition A.

4. ***S+/S- sequentially presented.*** During this condition, the S- was added. One stimulus was presented at a time (sequential presentation). For the first ten trials, S+ and S- alternated; thereafter, the presentations of S+ and S- were in semi-random order (Fellows, 1967). A total of 43 trials were conducted using this condition (10 in alternating order and 33 in random order). The cuttlefish was rewarded in the same way as in Condition A.

5. ***Shaping with food hopper.*** The food hopper was inserted into the tank and the cuttlefish received 9 food presentations alone, without the stimuli, to acclimate to receiving food in the hopper.

6. ***S+ with food hopper.*** 44 trials were conducted using only the S+, to further acclimate the cuttlefish to the food hopper. The S+ was positioned either to the left or the right of the food hopper, in semi-random order (Fellows, 1967). The cuttlefish was rewarded using the food hopper.

7. ***S+/S- simultaneously presented with food hopper.*** 52 trials were conducted using both stimuli (S+ and S-) simultaneously. The S+ was presented on either the left or the right side in semi-random order (Fellows, 1967). The cuttlefish was rewarded using the food hopper and the last 16 trials were video recorded using a camera placed above the housing tank.

8. ***S+ presented on computer monitor.*** 75 trials were conducted using only the S+, which was presented in the center of the computer monitor. The last half of these trials was video recorded.

9. ***S+/S- sequentially presented on computer monitor.*** For this condition, S+ and S- were presented sequentially and alternately. As in the shaping trials, above, the stimuli were presented in the center of the computer monitor. The cuttlefish was rewarded using the food hopper. A total of 15 trials were administered; all were video recorded.

10. ***S+/S- simultaneously presented on computer monitor.*** The two stimuli (S+ and S-) were presented simultaneously. S+ was presented on either the left or right side in semi-random order (Fellows, 1967). A total of 56 trials were administered; all trials were video recorded.

A summary of training procedures is presented in Table 1.

After data collection, the percent of successful trials for each condition were calculated. Chi-square tests were used to evaluate differences in responses to the S+ and S- and between methods of presentation.

RESULTS

The cuttlefish successfully discriminated between the physical objects (S+ and S-) when presented either sequentially (4. S+/S- sequentially presented: $X^2 = 12.765$, d.f. = 1, $p < 0.001$) or simultaneously (7. S+/S- simultaneously presented with food hopper: $X^2 = 13.37$, d.f. = 1, $p < 0.001$). Learning transferred readily to the computer images: the cuttlefish was 100% successful in sequential

Table 1. Training conditions and response times for the single *Sepia officinalis* subject.

	Description	Location of S	N	N trials	% Strike or % Correct	Average Response Time	SEM for Response Time
1.	S+ with crab	inside the training tank	~10 trials	10	---		
2.	S+ with crab	outside the tank	45	55	0.96	16.05	2.34
3.	S+ alone	outside the tank	45	100	0.87	30.04	2.97
4.	S+/S- sequential	outside the tank	43	143	0.66		
	S+		23		0.87	22.55	3.61
	S-		20		0.55	37.43	4.83
5.	food hopper alone		9	152	1.00	30.56	5.85
6.	S+ w/ food hopper	outside the tank	44	196	0.59	42.79	2.80
7.	S+/S- simultaneous	outside the tank	52	248	0.73	34.46	2.30
8.	S+	computer monitor	75	323	0.92	28.83	1.65
9.	S+/S- sequential	computer monitor	15	338	1.00		
	S+		8		1.00	23.75	3.29
	S-		7		0.00	60.00	0.00
10.	S+/S- simultaneous	computer monitor	56	394	0.86	30.50	2.37

presentations (9. S+/S- sequentially presented 0.001) and 86% successful in simultaneous on computer monitor: X^2 = 15.29, d.f. = 1, p <presentations (10. S+/S- simultaneously presented on computer monitor: X^2 = 28.78, d.f. = 1, $p < 0.001$). Average response times for each condition are shown in Table 1.

The cuttlefish successfully transferred their learning from the physical objects to the computer images. In fact, the computer images yielded a higher success rate for both sequential (S+ and S- presented sequentially: physical stimuli 66%, computer images 100 %, X^2 = 44.83, d.f. = 1, $p < 0.001$) and simultaneous (S+ and S- presented simultaneously: physical stimuli 73%, computer images 86%, X^2 = 20.94, d.f. = 1, $p < 0.001$) presentations. A comparison between the presentations of physical stimuli and computer images is shown in Figure 4.

DISCUSSION

Results show that the cuttlefish was able to transfer discrimination learning from physical stimuli to images of stimuli presented on a CRT monitor. To the best of my knowledge, this is the first time that this ability has been demonstrated in cephalopods. In addition, the cuttlefish responded more quickly and accurately to the presentations on the computer screen than she did to the physical stimuli. This result is likely due to the nature of incremental learning: learning typically increases as a function of the number of trials. In this experiment, the incremental learning continued even after stimuli were presented in a new way -- on the computer screen. This result implies that the cuttlefish did not experience a substantial difference between the physical stimuli and the computer images of the stimuli.

This research also investigated the feasibility of using a touch screen, which can

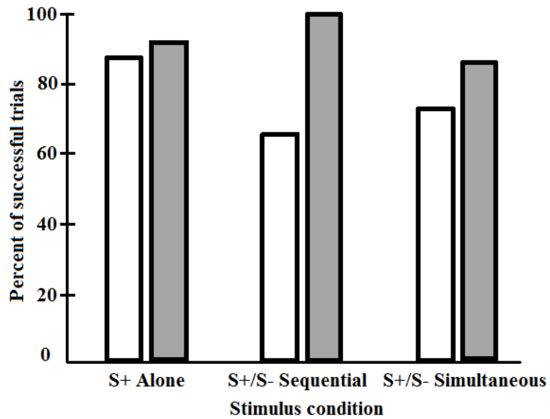

Figure 5. Percent of successful trials when stimuli were presented physically (open bars) or on a CRT monitor (shaded bars); S+ alone (conditions 3 & 6), S+/S- sequential (conditions 4 & 9), and S+/S- simultaneous (conditions 7 & 10). Asterisks indicate a significant difference (P<0.001).

display the stimuli and allow for response by touch. During both the physical and computer presentation conditions, the cuttlefish readily adapted to touching the Plexiglas in front of the S+ and to using a food hopper. These abilities are prerequisites to building a species-appropriate automated apparatus for testing discrimination-based learning.

To demonstrate species-typical behavior, a sample size of one is clearly inadequate; however, in this experiment, I was simply trying to determine if a cuttlefish is similar to vertebrates in its ability (1) to discriminate images presented on a CRT, and (2) be trained to touch an inert surface and then turn to receive a food reward in a separate hopper. If this particular cuttlefish had been unable to perform these tasks, the results would have been difficult to interpret; however, her success demonstrates that these actions are within the capabilities of this species. Further research with multiple subjects will be necessary to establish that this method is an efficient way to train cuttlefish and possibly other cephalopods, such as *Octopus*.

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